Archives of Disease in Childhood, 1970, **45**, 491.

Differential Protein Clearances in Indian Children with the Nephrotic Syndrome

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Chandra, R. K., Manchanda, S. S., Srivastava, R. N., and Soothill, J. F. (1970). *Archives of Disease in Childhood*, **45**, 491. **Differential protein clearances in Indian children with the nephrotic syndrome.** Differential clearances of five plasma proteins were studied in 37 north Indian children with the nephrotic syndrome, and these, and the serum β_{1C} levels were related to steroid response and, in some, renal biopsy histology. 16 children (43%) did not respond completely to steroids and a majority of them had a poorly selective proteinuria. The differential protein clearances suggest that these patients are a heterogeneous group. The serum complement β_{1C} levels in nephrotics were significantly lower than in control Indian and European children.

In most communities the majority of children with the nephrotic syndrome are responsive to steroids (93% in the series reported by Arneil and Lam (1966)) and have only minimal abnormality of renal biopsy histology (Cameron and White, 1965; Cameron, 1968), though there is always a minority of steroid resistant patients who often show abnormality of glomerular structure. In Nigeria, however, the situation is quite different; the majority of patients there do not respond to steroids and have abnormal renal biopsies, and there is epidemiological evidence that this difference arises, at least in part, from the high incidence of a form of nephritis associated with *P. malariae* infection (Gilles and Hendrickse, 1963; Hendrickse and Gilles, 1963; Soothill and Hendrickse, 1967).

Differential protein clearances, a quantitative measurement of the type of glomerular damage associated with heavy proteinuria (Hardwicke and Soothill, 1967), provide a means of identifying the steroid sensitive minority in European adult patients and Nigerian children with the nephrotic syndrome (Blainey *et al.*, 1960; Soothill and Hendrickse, 1967), and the steroid sensitive majority in European children (Cameron and White, 1965), all of whom have highly selective proteinuria, i.e. the clearance of larger proteins is very much less than the clearance of small proteins. The poorly selective group in European adults is clearly heterogeneous, both in terms of the protein clearance measurements, the underlying disease process, and the histology of the kidney (Hardwicke and Soothill, 1967), but it is difficult to study this in European nephrotic children, as poorly selective proteinuria is relatively rare, while the findings in Nigerian children define two predominant, remarkably homogeneous disease processes—the minority highly selective, and the majority with a characteristic discontinuity of linearity of selectivity of proteinuria (Soothill and Hendrickse, 1967). For this reason, studies are required of other populations of nephrotic children, in which poor response to steroids occurs fairly frequently and in which *P. malariae* is not a prominent pathogen. Children in Delhi with the nephrotic syndrome represent one such, and we report findings of differential protein clearances, renal biopsy, and steroid response in them.

Materials and Methods

Thirty-seven children, aged between 2½ years and 12 years, who attended the clinics at New Delhi or Amritsar, with the nephrotic syndrome (generalized oedema and proteinuria >0·1 g./kg. per day) in the period January 1968 to November 1969 were studied. Five of these children had previously been treated with steroids without significant change in the clinical condition or proteinuria; these are indicated in Table I. Since both the clinics are in teaching hospitals, it is possible that some degree of selection towards more severe cases may occur, particularly at the New Delhi clinic, but we are not aware that this was the case, or

Received 20 January 1970.

indeed how it could be done, apart from the initial use of steroids at home, which was done in only the 5 mentioned above. 9 patients were mildly hypertensive and 8 showed a moderate increase in blood urea. A past history suggestive of the acute nephritis syndrome was elicited in 2. All children were treated with prednisolone 2 mg./kg. per 24 hours and supportive measures. Treatment was maintained at this dose for 4 weeks if a response occurred, or for 8 weeks, if proteinuria persisted, when the response was classified as 'good' if random specimens of urine were negative for protein by the heat coagulation test, 'poor' if the test was still strongly positive (+++), and 'fair' if small amounts of proteinuria (tr,−,++) were detected. Percutaneous renal biopsy was performed in 15 children. The slides were classified as normal, 'minimal change', proliferative glomerulonephritis, or membranous glomerulonephritis by one of us (R.N.S.) who had no knowledge of the therapeutic outcome.

Differential protein clearances and serum β_{1C} were estimated by the double diffusion method described by Soothill (1962). Urine and serum samples, collected before treatment was started, were preserved with 1 drop of 0·1% sodium azide and stored at −20 °C. The specific antisera to albumin (A), siderophilin (S), IgG, β_{1C}, and α-macroglobulin (αM) were raised in rabbits or sheep. The estimations were made after the patients had been classified therapeutically and histologically.

Reactions of identity were shown between each of the proteins studied in the urine and the serum, by the double gel diffusion technique. Serum β_{1C} concentrations are expressed as a percentage of the M.R.C. Research Standard Serum for immunoglobulins G, A, and M, and values for 15 healthy Indian children aged 2 to 12 years, estimated by the same technique, and for 19 European children (Ngu and Soothill, 1969) using a single diffusion technique, but the same standard, are also shown.

TABLE I

Differential Protein Clearances Expressed as Percentage of Albumin Clearance in Different Treatment Response Groups

Response	Biopsy	C_S/C_A (%)	C_{IgG}/C_A (%)	$C_{\beta_{1C}}/C_A$ (%)	$C_{\alpha M}/C_A$ (%)	Serum β_{1C} (%)	Previous Steroid Treatment
Good (21)	Normal	85	16	9	<0·20	112	
	Normal	95	11	4·5	<0·12	75	
	Minimal change	90	20	12	0·25	87	
	Normal	85	8	4	<0·20	87	
	Minimal change	95	16	12	<0·20	112	
	—	105	12	8	0·12	87	
	—	75	30	23	<0·24	75	
	—	88	16	7	<0·21	137	
	—	96	33	12	0·4	87	
	—	98	6	7	<0·05	25	
	—	105	11	9	0·15	62	
	—	97	30	12	<0·04	87	
	—	73	8	7	0·10	75	
	—	92	7	11	<0·20	62	
	—	90	5	5	0·05	125	
	—	110	16	4	0·15	37	
	—	85	9	5	0·12	87	
	—	95	5	2	<0·20	62	
	—	90	10	5	0·16	87	
	—	102	13	7	<0·20	62	
	—	102	6	3	0·14	112	
Fair (8)	Prolif.	95	23	12	0·9	25	
	Prolif.	90	18	12	0·25	62	+
	Minimal change	95	23	17	0·4	75	
	Normal	80	20	7	0·4	62	
	Membranous	90	40	12	0·8	87	+
	—	65	45	12	0·9	112	
	—	103	33	12	0·4	37	
	—	95	33	7	0·9	87	
Poor (8)	Membranous	80	50	20	0·9	87	
	—	92	55	16	2·0	50	+
	Prolif.	105	50	14	0·7	50	
	Minimal change	95	40	14	0·8	150	+
	Prolif.	92	42	29	1·4	37	+
	—	70	50	28	0·8	62	
	—	95	50	14	0·25	37	
	—	105	33	4·5	1·5	62	

— = Not done.

Results

Of the 37 children, 21 (57%) responded fully to 4–8 weeks of prednisolone treatment. If the 5 patients who were referred after some steroid treatment are excluded, this incidence rises to 21 out of 32–66%. These figures confirm previous impressions that steroid response is less frequent in Indian nephrotic children than in some populations.

In Table I the renal biopsy findings, differential protein clearance results, and serum β_{1C} levels are given for the patients, classified according to their response to steroid treatment as good, fair, or poor. The mean value for each group of the clearances of each protein expressed as a percentage of the clearance of albumin is plotted against the molecular weight of each protein in Fig. 1. For all proteins, except siderophilin, which is passed nearly as readily as albumin, the selectivity is greatest in the good responders, and least in the poor responders. Each of these distinctions is significant by t test analysis of log $\frac{C_{IgG}}{C_{Alb}}$ % (Table II). Similar separations are achieved by β_{1C} and α_M, but analysis for α_M is difficult because of threshold values, though the differences are obvious, and perhaps achieve the clearest separation, since all the good responders had C_α/C_A of 0·4 or less, and all the rest had $C_{\alpha M}/C_A$ of 0·4 or greater.

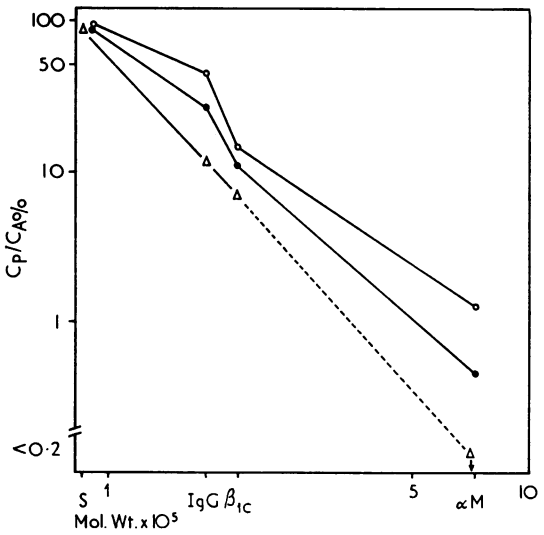

FIG. 1.—*Mean value of clearances of siderophilin (S), IgG, β_{1C}, and α macroglobulin (α_M) expressed as percentage of clearance of albumin (A), and plotted against the molecular weight of the protein, for Indian children with nephrotic syndrome, classified by steroid response as good (△), fair (●), and poor (○).*

TABLE II

C_{IgG}/C_A in Indian Nephrotic Children Classified by Steroid Response and by Renal Biopsy Histology

Group	No.	Log Mean (%)	t	p
Good response	21	11	10·55	<0·001
Fair response	8	27	3·21	<0·01
Poor response	8	46		
Minimal change	8	16	4·04	<0·01
Histological abnormality	7	37		

This difference of discrimination by the different proteins arises from the heterogeneity of linearity on these plots for different individuals, which is illustrated in Fig. 2, in which the results of 5 patients selected to illustrate this point are plotted. 3 give essentially linear plots, of different selectivities, whereas 2 others were poorly selective for IgG but highly selective for α_M. We do not have evidence for the reproducibility of this in these children, though such evidence has been reported previously (Hardwicke *et al.*, 1970).

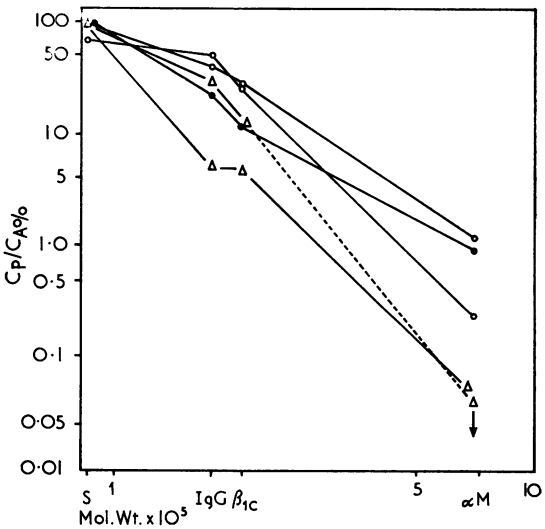

FIG. 2.—*Differential protein clearance results, plotted as in Fig. 1, for 5 selected Indian children with the nephrotic syndrome, 3 of whom showed proteinuria of a range of selectivities which resulted in approximately linear plots, and 2 of whom show marked discontinuity of linearity.*

Similar relations are shown between differential protein clearances and renal biopsy histology. The histology also clearly was related to treatment response, since all the 5 patients with good response who were biopsied had essentially normal renal histology, whereas this was true of only 3 of the 10 who did not respond fully. The numbers were small, so comparison between those classified as membranous nephritis, and those classified as proliferative cannot be made statistically, but C_{IgG}/C_A is significantly higher for them together than it is for the patients with minimal renal histological abnormality (Table II), and similar trends are shown for the other proteins.

The serum β_{1C} values in the healthy Indian children (log mean 110%) were remarkably similar to those of healthy English children (log mean 108%) (Fig. 3), and the Indian nephrotic children

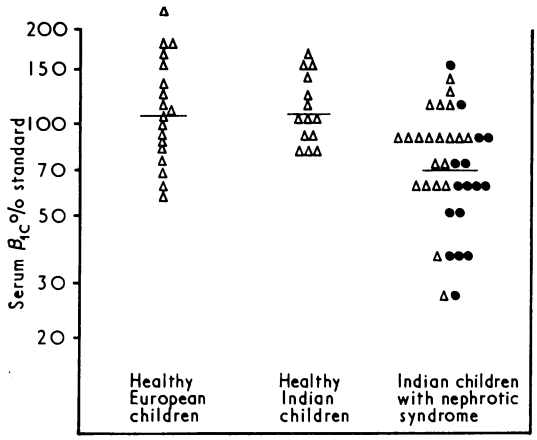

FIG. 3.—*Serum β_{1C} concentration in Indian children with the nephrotic syndrome (△ = good steroid response, ● = fair or poor steroid response), in healthy Indian children, and in healthy European children (from Ngu and Soothill, 1969) expressed as percentage of M.R.C. standard serum for immunoglobulins G, A, and M. The log mean of each group is indicated.*

gave values (log mean 74%) which were significantly lower than the healthy Indian children ($t = 61 \cdot 2$; $P = < 0 \cdot 001$). There were trends for the good responders, and for the patients with minimal renal histological abnormality to give higher values, but these were not significant.

Discussion

Nephrotic syndrome is the clinical manifestation of glomerular damage, resulting in a large leak of protein, and there are many causes of it (Hardwicke and Soothill, 1967). The prognosis and treatment response of any particular patient are related to the underlying renal lesion rather than to the apparent severity of the presenting symptoms. A good correlation between the short-term steroid response and the selectivity of differential protein clearances has been shown for European adults (Blainey *et al.*, 1960), European children (Cameron and White, 1965; Cameron, 1968), and Nigerian children (Soothill and Hendrickse, 1967). Our observations confirm this for another population group—Indian children. Probably good response to steroids is rarer in this population than in nephrotic children in Europe, and more common than in nephrotic children in Nigeria, and this is reflected in the proportion with highly selective proteinuria, and by the renal biopsy histology, though the correlation between these different findings is not perfect. It is likely that the difference in the proportion of nephrotic children with poor response and a poorly selective protein excretion would differ from one population group to another, depending upon the incidence of different causes of the glomerular damage in the different populations. In Nigeria, epidemiological evidence suggests that infection with *P. malariae* is an important cause of the childhood nephrotic syndrome (Gilles and Hendrickse, 1963; Hendrickse and Gilles, 1963), and this accounts for the fact that the steroid responsive group are a minority with another common illness superimposed. In Delhi, malaria has been almost unknown for the past 5 years, and it is most unlikely that malarial infection is a significant contributory cause for the relatively large number of poor and fair responders among Delhi nephrotic children. It is possible that this incidence represents an allergic reaction to one of the other infectious diseases, still endemic in India, though we do not know what.

The use of different means of expression of the differential protein clearance data has been debated (Hardwicke *et al.*, 1970). Where a line can be drawn through a series of points, slope statistics are attractive (Joachim *et al.*, 1964), but clearly this is possible in only some of our patients (Fig. 2), though probably in a higher proportion than in the Nigerian children. As approximations to this, implicitly assuming such linearity, C_{IgG}/C_S (Cameron and Blandford, 1966), or $C_{\alpha M}/C_A$ (MacLean and Robson, 1967) have been advocated, and our data are probably consistent with the view of the latter workers, that theirs is the best single predictor of steroid response, though the former has practical advantages, and both eliminate some potentially useful information. The discontinuity of linearity (or dog's leg proteinuria) leading to this complexity provides evidence of heterogeneity in the poor

steroid responders, which is apparently greater in our patients than that in Nigerian nephrotic children (Soothill and Hendrickse, 1967). This perhaps points to multiple aetiology in our patients.

The serum complement component β_{1c} levels in normal Indian children are remarkably similar to those in a European population. In our patients with the nephrotic syndrome, there was a significant depression of serum β_{1c} concentration which involved both those with good and poor steroid response since, though there was a tendency for those with histological abnormality and for the poor responders to give lower values, this small trend was not significant. Though there has been debate about serum complement levels in steroid sensitive nephrotic syndrome, and there is evidence that it may be an immunological disease (Lange *et al.*, 1957; Ngu, Barratt, and Soothill, 1970), the latter found no difference between the serum β_{1c} levels in European steroid sensitive relapsing nephrotic syndrome, and in the healthy European children whose data are plotted in Fig. 3. It is possible that there is a regional difference even in the steroid sensitive group, though the concentration of a plasma protein in the face of heavy proteinuria may well be influenced by many non-specific variables which may differ from place to place.

We are grateful to Drs. D. Myekar and K. K. Khanna for collection of some of the samples, and to Professor O. P. Ghai for permission to publish this paper. The study was done during the tenure of a Commonwealth Medical Fellowship to one of us (R.K.C.).

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